Cleavage Close to the End of DNA Fragments (oligonucleotides)

To test the varying requirements restriction endonucleases have for the number of bases flanking their recognition sequences, a series of short, double-stranded oligonucleotides that contain the restriction endonuclease recognition sites (shown in red) were digested. This information may be helpful when choosing the order of addition of two restriction endonucleases for a double digest (a particular concern when cleaving sites close together in a polylinker), or when selecting enzymes most likely to cleave at the end of a DNA fragment.

The experiment was performed as follows: 0.1 A_{260} unit of oligonucleotide was phosphorylated using T4 polynucleotide kinase and γ-[^{32}P] ATP. 1 µg of 5´ [^{32}P]-labeled oligonucleotide was incubated at 20°C with 20 units of restriction endonuclease in a buffer containing 70 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 5 mM DTT and NaCl or KCl depending on the salt requirement of each particular restriction endonuclease. Aliquots were taken at 2 hours and 20 hours and analyzed by 20% PAGE (7 M urea). Percent cleavage was determined by visual estimate of autoradiographs.

As a control, self-ligated oligonucleotides were cleaved efficiently. Decreased cleavage efficiency for some of the longer palindromic oligonucleotides may be caused by the formation of hairpin loops.

| A | B | C | E | H | K | M | N | P | S | X |

Enzyme	Oligo Sequence	Chain Length	% Cleavage	
			2 hr	20 hr
Acc I	GGTCGACC	8	0	0
	CGGTCGACCG	10	0	0
	CCGGTCGACCGG	12	0	0
Afl III	CACATGTG	8	0	0
	CCACATGTGG	10	>90	>90
	CCCACATGTGGG	12	>90	>90
Asc I	GGCGCGCC	8	>90	>90
	AGGCGCGCCT	10	>90	>90
	TTGGCGCGCCAA	12	>90	>90
Ava I	CCCCGGGG	8	50	>90
	CCCCCGGGGG	10	>90	>90
	TCCCCCGGGGGA	12	>90	>90
BamH I	CGGATCCG	8	10	25
	CGGGATCCCG	10	>90	>90
	CGCGGATCCGCG	12	>90	>90

Bgl II	CAGATCTG GAAGATCTTC GGAAGATCTTCC	8 10 12	0 75 25	0 >90 >90
BssH II	GGCGCGCC AGGCGCGCCT TTGGCGCGCCAA	8 10 12	0 0 50	0 0 >90
BstE II	GGGT(A/T)ACCC	9	0	10
BstX I	AACTGCAGAACCAATGCATTGG AAAACTGCAGCCAATGCATTGGAA CTGCAGAACCAATGCATTGGATGCAT	22 24 27	0 25 25	0 50 >90
Cla I	CATCGATG GATCGATC CCATCGATGG CCCATCGATGGG	8 8 10 12	0 0 >90 50	0 0 >90 50
EcoR I	GGAATTCC CGGAATTCCG CCGGAATTCCGG	8 10 12	>90 >90 >90	>90 >90 >90
Hae III	GGGGCCCC AGCGGCCGCT TTGCGGCCGCAA	8 10 12	>90 >90 >90	>90 >90 >90
Hind III	CAAGCTTG CCAAGCTTGG CCCAAGCTTGGG	8 10 12	0 0 10	0 0 75
Kpn I	GGGTACCC GGGGTACCCC CGGGGTACCCCG	8 10 12	0 >90 >90	0 >90 >90
Mlu I	GACGCGTC CGACGCGTCG	8 10	0 25	0 50
Nco I	CCCATGGG CATGCCATGGCATG	8 14	0 50	0 75
Nde I	CCATATGG CCCATATGGG CGCCATATGGCG GGGTTTCATATGAAACCC GGAATTCCATATGGAATTCC GGGAATTCCATATGGAATTCCC	8 10 12 18 20 22	0 0 0 0 75 75	0 0 0 0 >90 >90

Nhe I	GGCTAGCC	8	0	0
	CGGCTAGCCG	10	10	25
	CTAGCTAGCTAG	12	10	50
Not I	TTGCGGCCGCAA	12	0	0
	ATTTGCGGCCGCTTTA	16	10	10
	AAATATGCGGCCGCTATAAA	20	10	10
	ATAAGAATGCGGCCGCTAAACTAT	24	25	90
	AAGGAAAAAAGCGGCCGCAAAAGGAAAA	28	25	>90
Nsi I	TGCATGCATGCA	12	10	>90
	CCAATGCATTGGTTCTGCAGTT	22	>90	>90
Pac I	TTAATTAA	8	0	0
	GTTAATTAAC	10	0	25
	CCTTAATTAAGG	12	0	>90
Pme I	GTTTAAAC	8	0	0
	GGTTTAAACC	10	0	25
	GGGTTTAAACCC	12	0	50
	AGCTTTGTTTAAACGGCGCGCCGG	24	75	>90
Pst I	GCTGCAGC	8	0	0
	TGCACTGCAGTGCA	14	10	10
	AACTGCAGAACCAATGCATTGG	22	>90	>90
	AAAACTGCAGCCAATGCATTGGAA	24	>90	>90
	CTGCAGAACCAATGCATTGGATGCAT	26	0	0
Pvu I	CCGATCGG	8	0	0
	ATCGATCGAT	10	10	25
	TCGCGATCGCGA	12	0	10
Sac I	CGAGCTCG	8	10	10
Sac II	GCCGCGGC	8	0	0
	TCCCCGCGGGGA	12	50	>90
Sal I	GTCGACGTCAAAAGGCCATAGCGGCCGC	28	0	0
	GCGTCGACGTCTTGGCCATAGCGGCCGCGG	30	10	50
	ACGCGTCGACGTCGGCCATAGCGGCCGCGGAA	32	10	75
Sca I	GAGTACTC	8	10	25
	AAAAGTACTTTT	12	75	75
Sma I	CCCGGG	6	0	10
	CCCCGGGG	8	0	10
	CCCCCGGGGG	10	10	50
	TCCCCCGGGGGA	12	>90	>90

Spe I	GACTAGTC	8	10	>90
	GGACTAGTCC	10	10	>90
	CGGACTAGTCCG	12	0	50
	CTAGACTAGTCTAG	14	0	50
Sph I	GGCATGCC	8	0	0
	CATGCATGCATG	12	0	25
	ACATGCATGCATGT	14	10	50
Stu I	AAGGCCTT	8	>90	>90
	GAAGGCCTTC	10	>90	>90
	AAAAGGCCTTTT	12	>90	>90
Xba I	CTCTAGAG	8	0	0
	GCTCTAGAGC	10	>90	>90
	TGCTCTAGAGCA	12	75	>90
	CTAGTCTAGACTAG	14	75	>90
Xho I	CCTCGAGG	8	0	0
	CCCTCGAGGG	10	10	25
	CCGCTCGAGCGG	12	10	75
Xma I	CCCCGGGG	8	0	0
	CCCCCGGGGG	10	25	75
	CCCCCCGGGGGG	12	50	>90
	TCCCCCCGGGGGGA	14	>90	>90

Cleavage Close to the End of DNA Fragments (linearized vector)

Linearized vectors were incubated with the indicated enzymes (10 units/µg) for 60 minutes at the recommended incubation temperature and NEBuffer for each enzyme. Following ligation and transformation, cleavage efficiencies were determined by dividing the number of transformants from the digestion reaction by the number obtained from religation of the linearized DNA (typically 100-500 colonies) and subtracting from 100%. "Base Pairs from End" refers to the number of double-stranded base pairs between the recognition site and the terminus of the fragment; this number does not include the single-stranded overhang from the initial cut. Since it has not been demonstrated whether these single-stranded nucleotides contribute to cleavage efficiency, 4 bases should be added to the indicated numbers when designing PCR primers. Average efficiencies were rounded to the nearest whole number; experimental variation was typically within 10%. The numbers in parentheses refer to the number of independent trials for each enzyme tested (from Moreira, R. and Noren, C. (1995), Biotechniques, 19, 56-59).

Note: As a general rule, enzymes not listed below require 6 bases pairs on either side of their recognition site to cleave efficiently.

| A | B | E | H | K | M | N | P | S | X |

Enzyme	Base pairs from End	%Cleavage Efficiency	Vector	Initial Cut
Aat II	3 2 1	88 (2) 100 (2) 95 (2)	LITMUS 29 LITMUS 28 LITMUS 29	Nco I Nco I PinA I
Acc65 I	2 1	99 (2) 75 (3)	LITMUS 29 pNEB193	Spe I Sac I
Afl II	1	13 (2)	LITMUS 29	Stu I
Age I	1 1	100 (1) 100 (2)	LITMUS 29 LITMUS 29	Xba I Aat II
Apa I	2	100 (1)	LITMUS 38	Spe I
Asc I	1	97 (2)	pNEB193	BamH I
Avr II	1	100 (2)	LITMUS 29	Sac I
BamH I	1	97 (2)	LITMUS 29	Hind III
Bgl II	3	100 (2)	LITMUS 29	Nsi I
BsiW I	2	100 (2)	LITMUS 29	BssH II
BspE I	2 1	100 (1) 8 (2)	LITMUS 39 LITMUS 38	BsrG I BsrG I
BsrG I	2 1	99 (2) 88 (2)	LITMUS 39 LITMUS 38	Sph I BspE I
BssH II	2	100 (2)	LITMUS 29	BsiW I
Eag I	2	100 (2)	LITMUS 39	Nhe I
EcoR I	1 1 1	100 (1) 88 (1) 100 (1)	LITMUS 29 LITMUS 29 LITMUS 39	Xho I Pst I Nhe I
EcoR V	1	100 (2)	LITMUS 29	Pst I
Hind III	3 2 1	90 (2) 91 (2) 0 (2)	LITMUS 29 LITMUS 28 LITMUS 29	Nco I Nco I BamH I
Kas I	2 1	97 (1) 93 (1)	LITMUS 38 LITMUS 38	NgoM IV Hind III
Kpn I	2 2	100 (2) 100 (2)	LITMUS 29 LITMUS 29	Spe I Sac I

	1	99 (2)	pNEB193	Sac I
Mlu I	2	99 (2)	LITMUS 39	Eag I
Mun I	2	100 (1)	LITMUS 39	NgoM IV
Nco I	2	100 (1)	LITMUS 28	Hind III
NgoM IV	2	100 (1)	LITMUS 39	Mun I
Nhe I	1 2	100 (1) 82 (1)	LITMUS 39 LITMUS 39	EcoR I Eag I
Not I	7 4 1	100 (2) 100 (1) 98 (2)	Bluescript SK- Bluescript SK- Bluescript SK-	Spe I Ksp I Xba I
Nsi I	3 3 2	100 (2) 77 (4) 95 (2)	LITMUS 29 LITMUS 29 LITMUS 28	BssH II Bgl II BssH II
Pac I	1	76 (3)	pNEB193	BamH I
Pme I	1	94 (2)	pNEB193	Pst I
Pst I	3 2 1	98 (1) 50 (5) 37 (3)	LITMUS 29 LITMUS 39 LITMUS 29	EcoR V Hind III EcoR I
Sac I	1	99 (2)	LITMUS 29	Avr II
Sal I	3 2 1	89 (2) 23 (2) 61 (3)	LITMUS 39 LITMUS 39 LITMUS 38	Spe I Sph I Sph I
Spe I	2 2	100 (2) 100 (2)	LITMUS 29 LITMUS 29	Acc65 I Kpn I
Sph I	2 2 1	99 (1) 97 (1) 92 (2)	LITMUS 39 LITMUS 39 LITMUS 38	Sal I BsrG I Sal I
Xba I	1 1	99 (2) 94 (1)	LITMUS 29 LITMUS 29	Age I PinA I
Xho I	1	97 (2)	LITMUS 29	EcoR I
Xma I	2 2	98 (1) 92 (1)	pNEB193 pNEB193	Asc I BssH II